MicroRNA Expression Profile and DNA Damage Response in Cultured Human Fibroblasts in Space

Honglu Wu
NASA Johnson Space Center

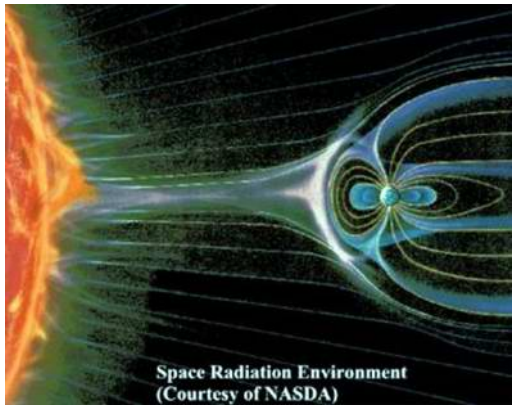
ASGSR Annual Meeting
Pasadena, California October, 25, 2014

Questions to be Addressed

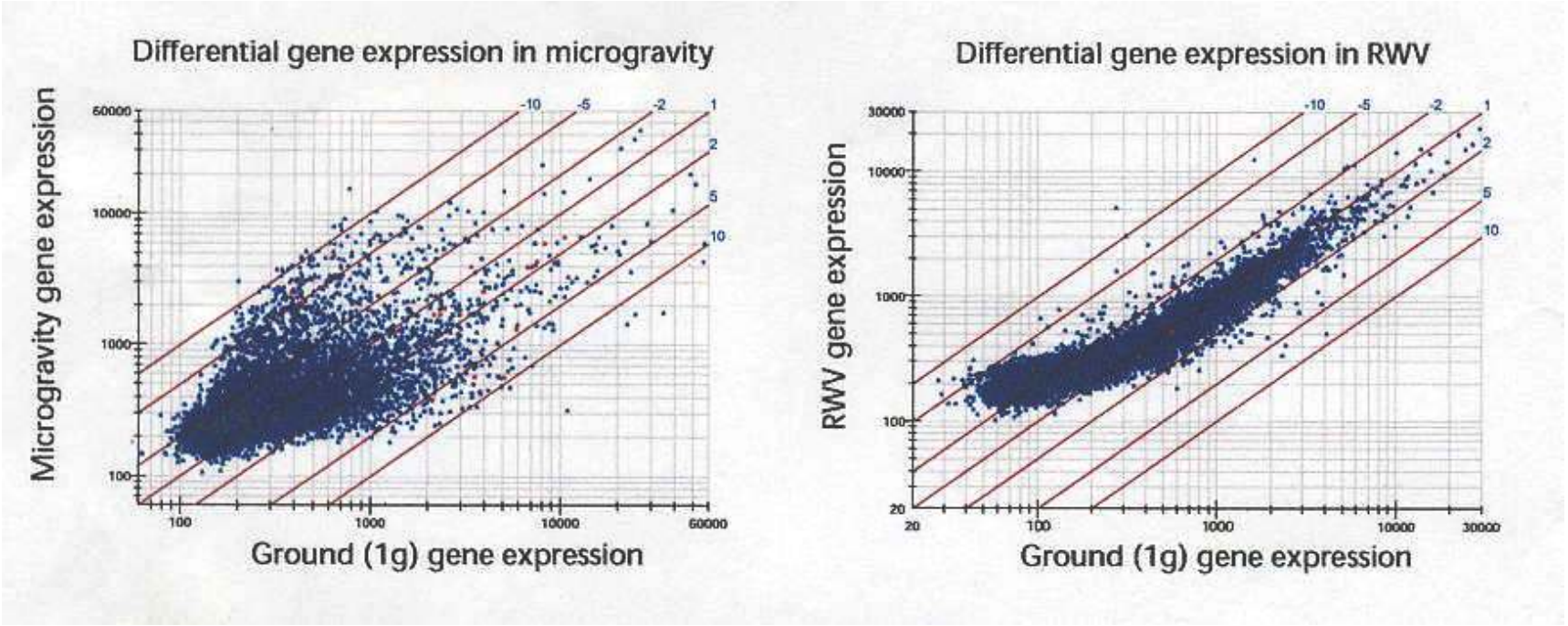
Does spaceflight influence RNA and miRNA expressions in non-dividing cultured cells?

Does microgravity affect cellular responses in living organisms to space radiation exposure?

Does microgravity and other spaceflight factors affect cellular responses to DNA damages?



Gene expression changes in space



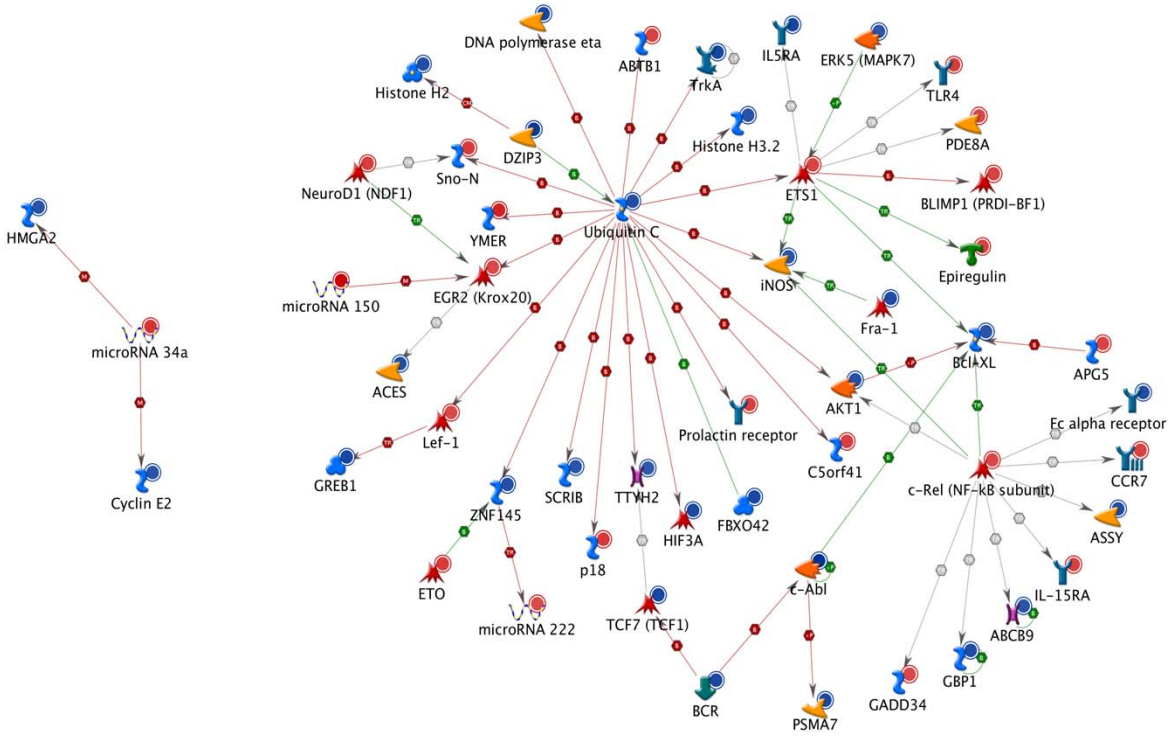
Hammond et al. Nature Medicine 1999

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 37, pp. 32483–32490, September 16, 2011
Printed in the U.S.A.

Effects of Simulated Microgravity on Expression Profile of MicroRNA in Human Lymphoblastoid Cells*[S]

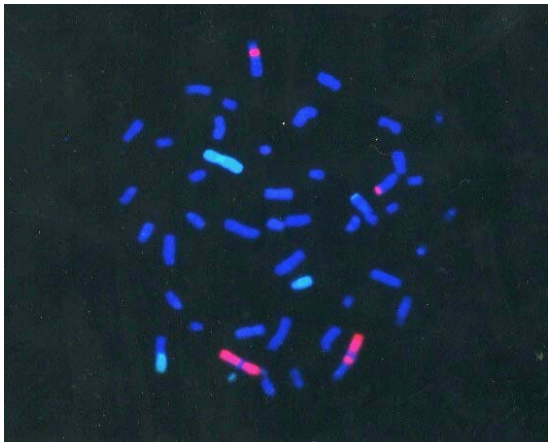
Received for publication, June 2, 2011, and in revised form, July 18, 2011 Published, JBC Papers in Press, July 20, 2011, DOI 10.1074/jbc.M111.267765

Lingegowda S. Mangala[‡§1,2], Ye Zhang[‡¶1], Zhenhua He[§], Kamal Emami[‡], Govindarajan T. Ramesh[‖], Michael Story[**], Larry H. Rohde[§], and Honglu Wu[‡]



The direct interaction analysis showed several projected networks with c-Rel, ETS1 and Ubiquitin C as key factors. Several genes showed direct interactions with miRNAs that were found to be altered in simulated microgravity environment. Seven genes cyclin E2, HMGA2, EGR2, ZNF145, Ubiquitin C, ETS1 and c-Rel were subjected to validation analysis using Quantitative Real-time PCR.

Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated in vitro with low-LET radiation
(Wu et al. Phys. Med. 2001)



Mission: STS-103

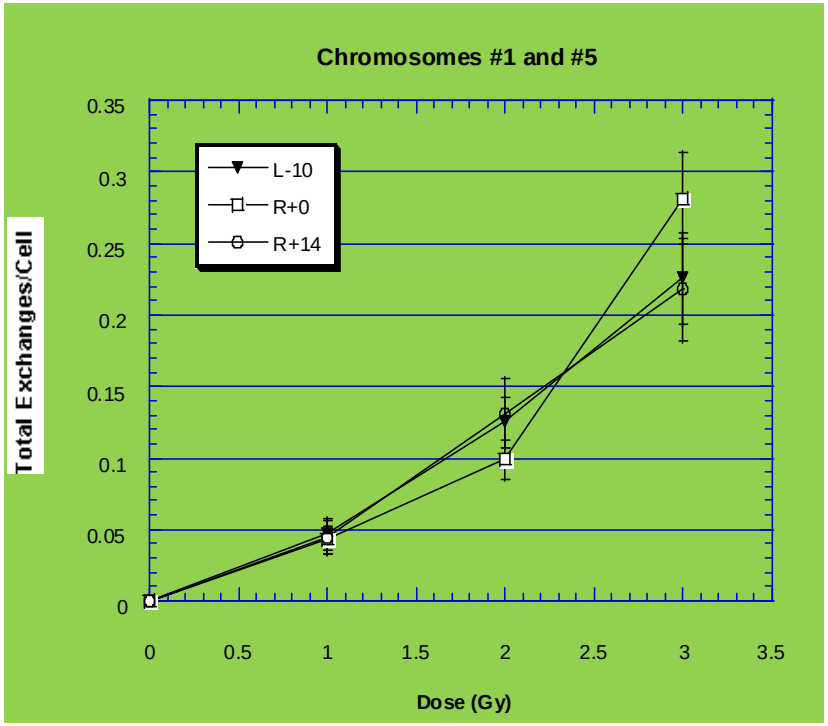
Duration: 8 days

Blood draw schedule: L-10, R+0 and R+14

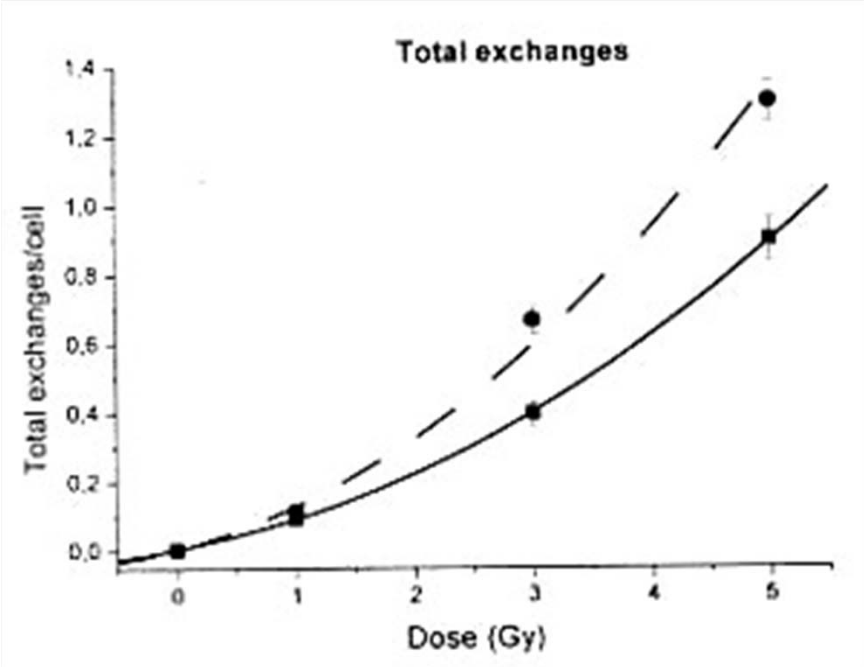
Irradiation: Whole blood was collected from the astronaut and irradiated with gamma rays

Chromosome analysis: Chromosomes #1 and #5 were painted.

Conclusion: No differences in radiosensitivity were found.

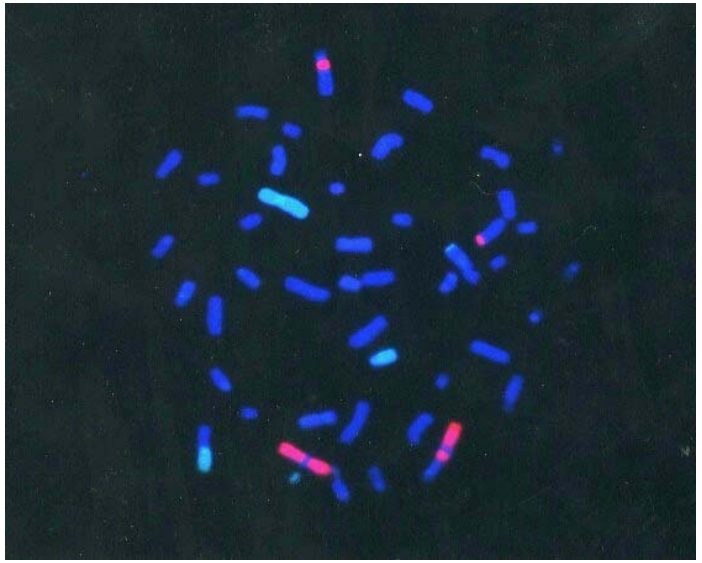


Greco et al. Adv. Space Res. 2003



Significant difference was found in the dose response curve between the pre- and post-flight samples.

- Cosmonaut's blood samples were collected 3 days after landing
- Samples were exposed to X-rays

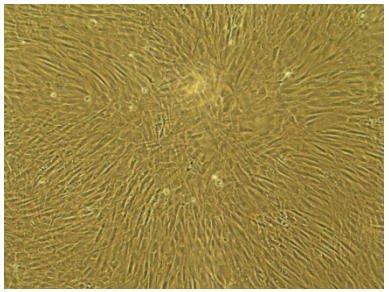


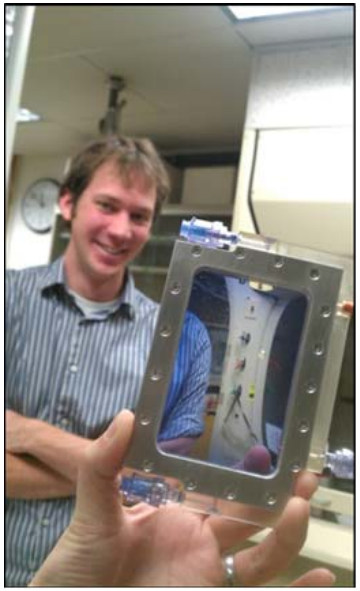
MICRO-7 FLIGHT EXPERIMENT

- Investigate changes of RNA and miRNA expressions in G1 human fibroblast cells in space
- Investigate the cellular response to bleomycin-inuced DNA damages in Ge human fibroblast cells in space

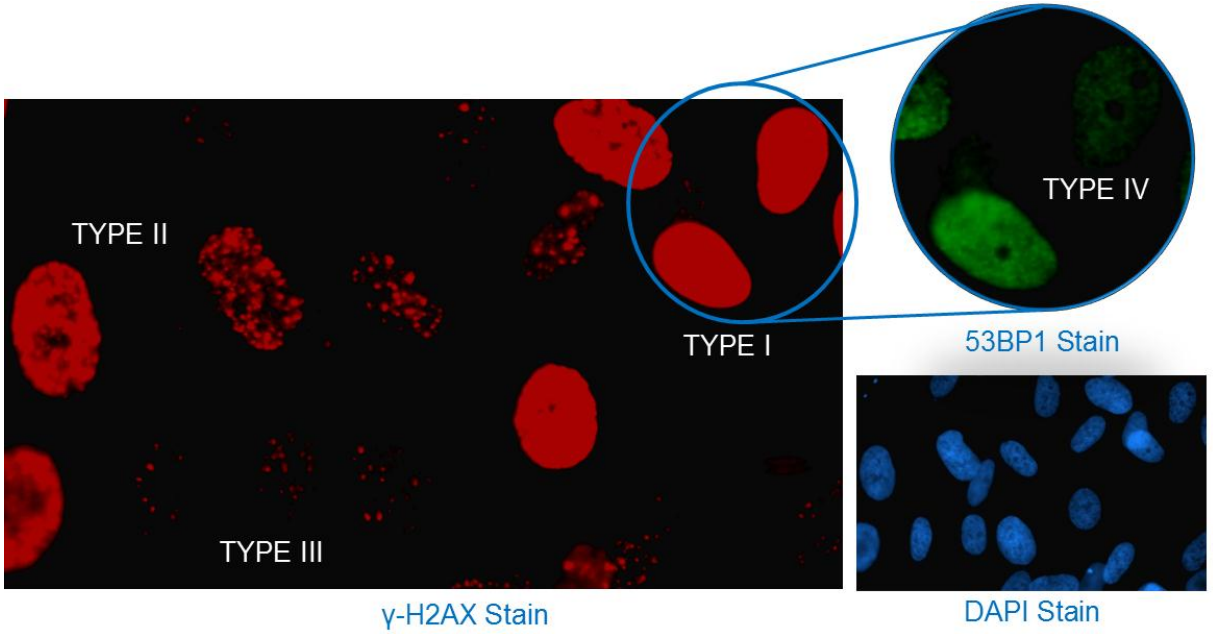
Our Micro-7 experiment was launched in April, 2014

Human fibroblasts

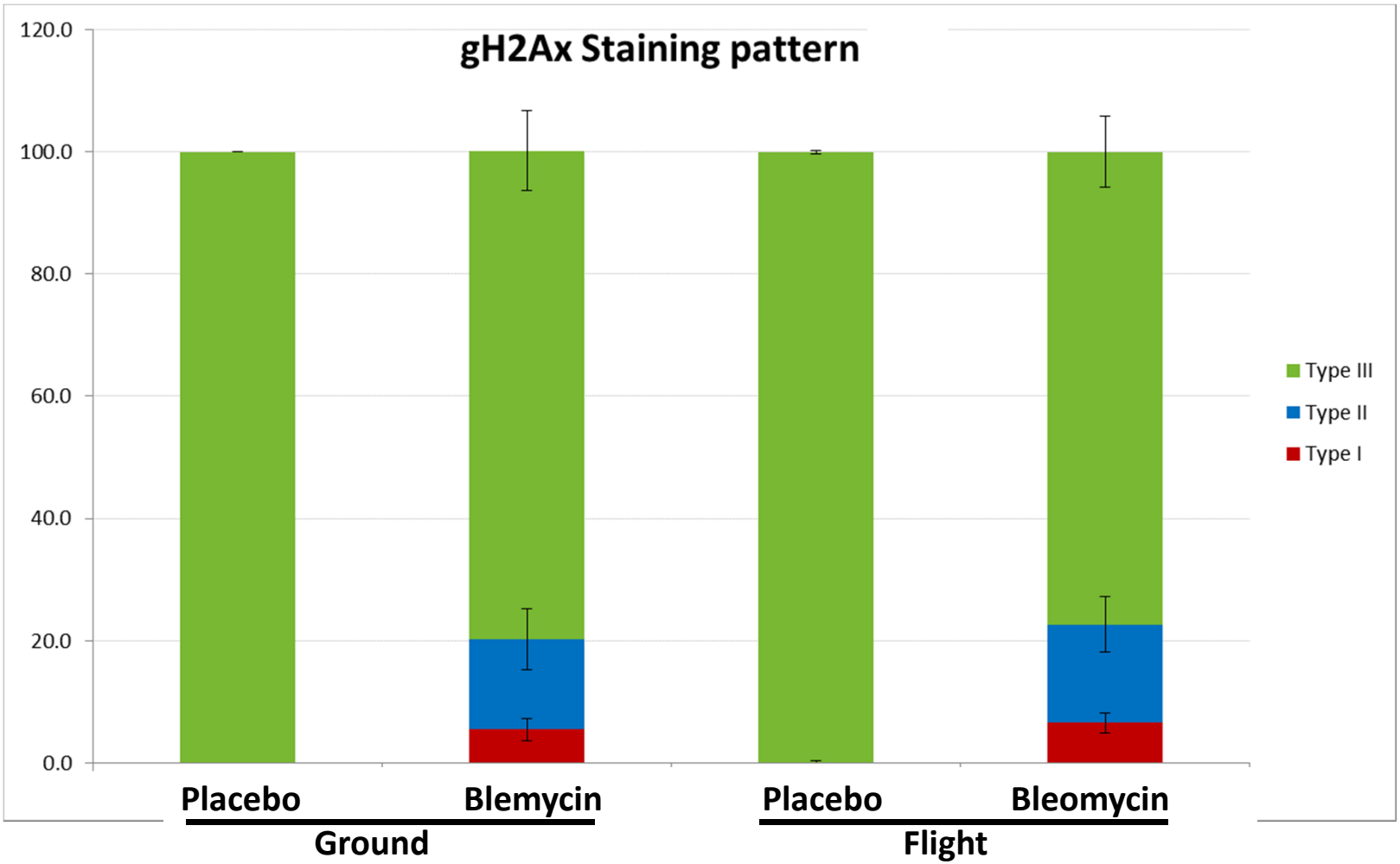


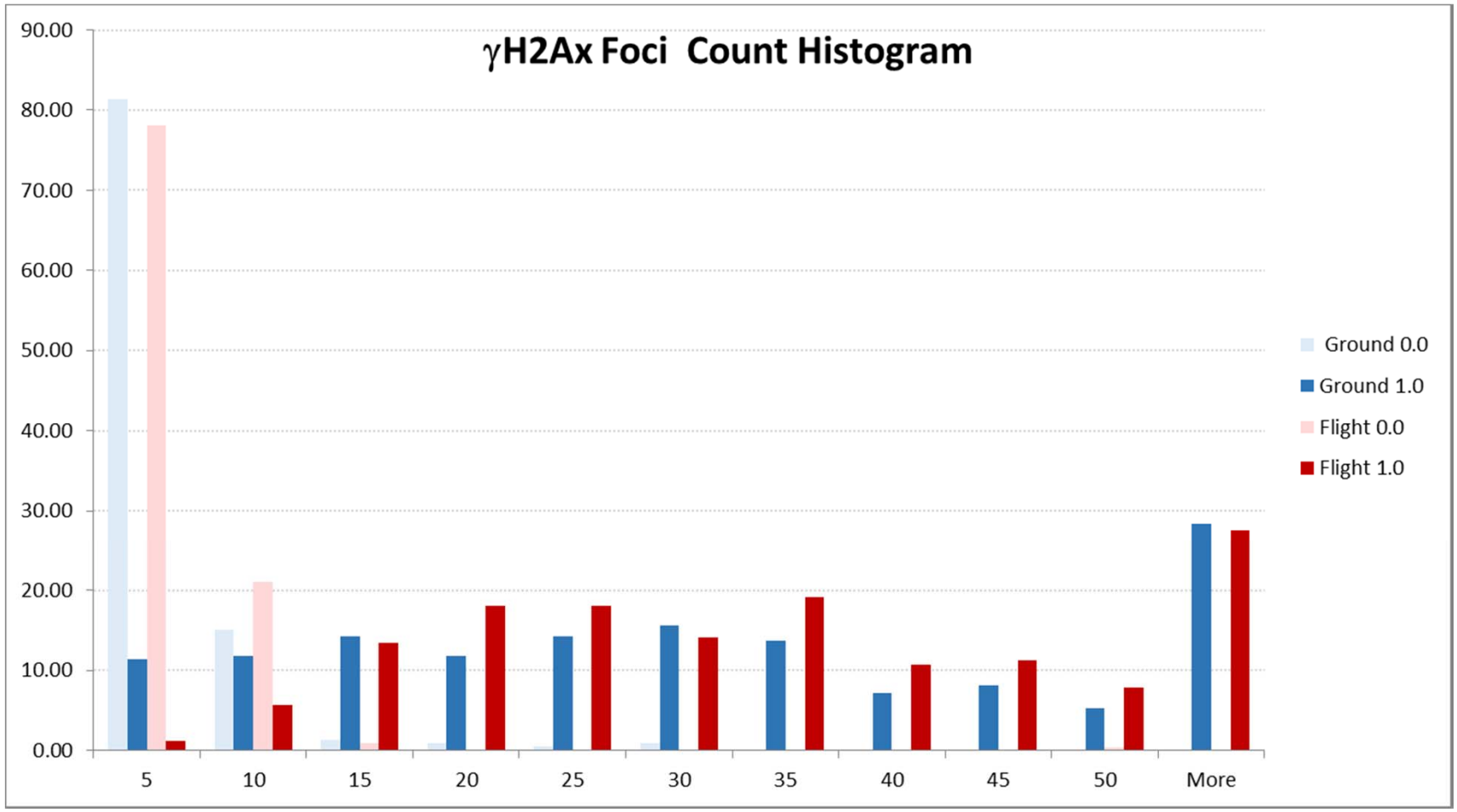


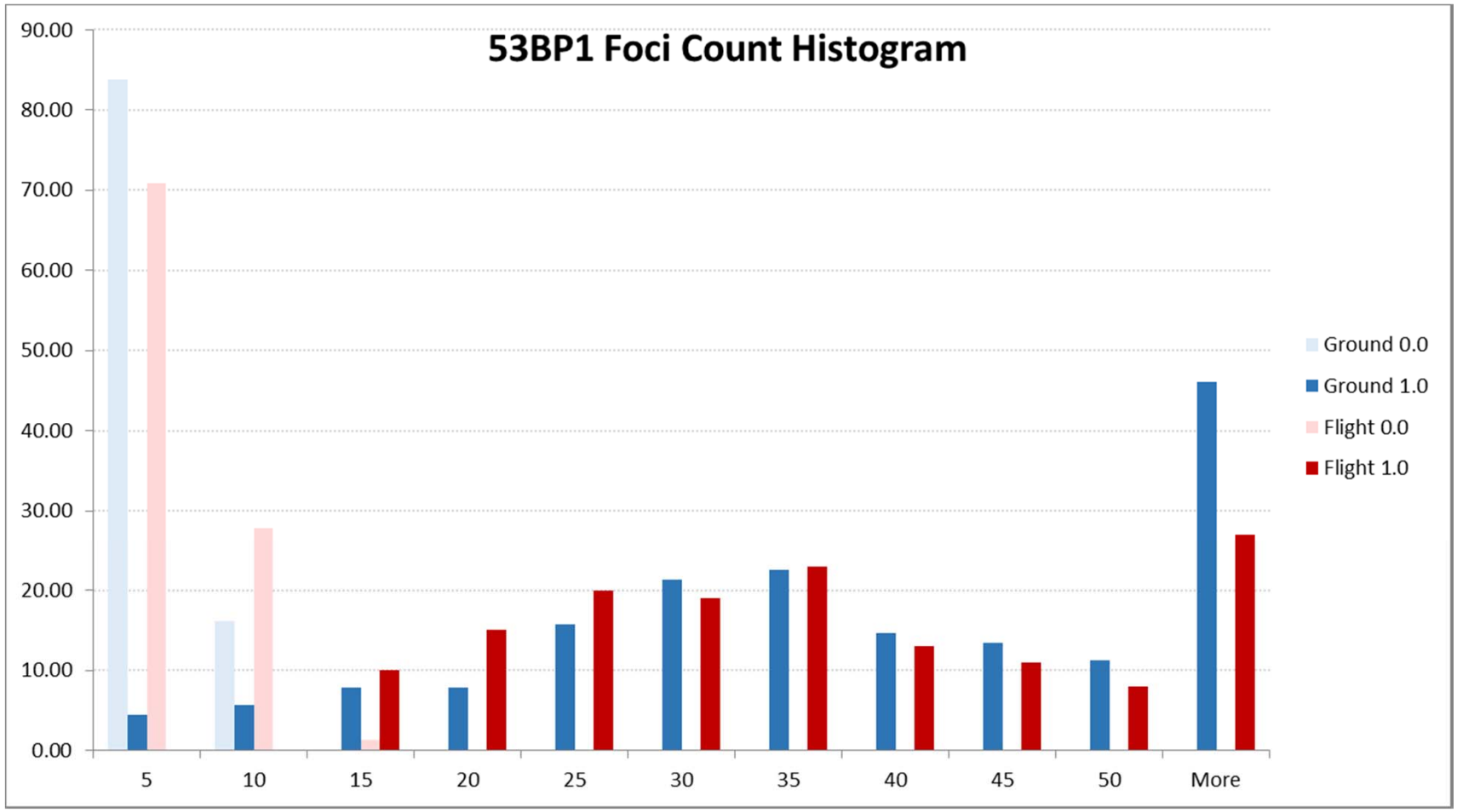
BioCell from Bioserve

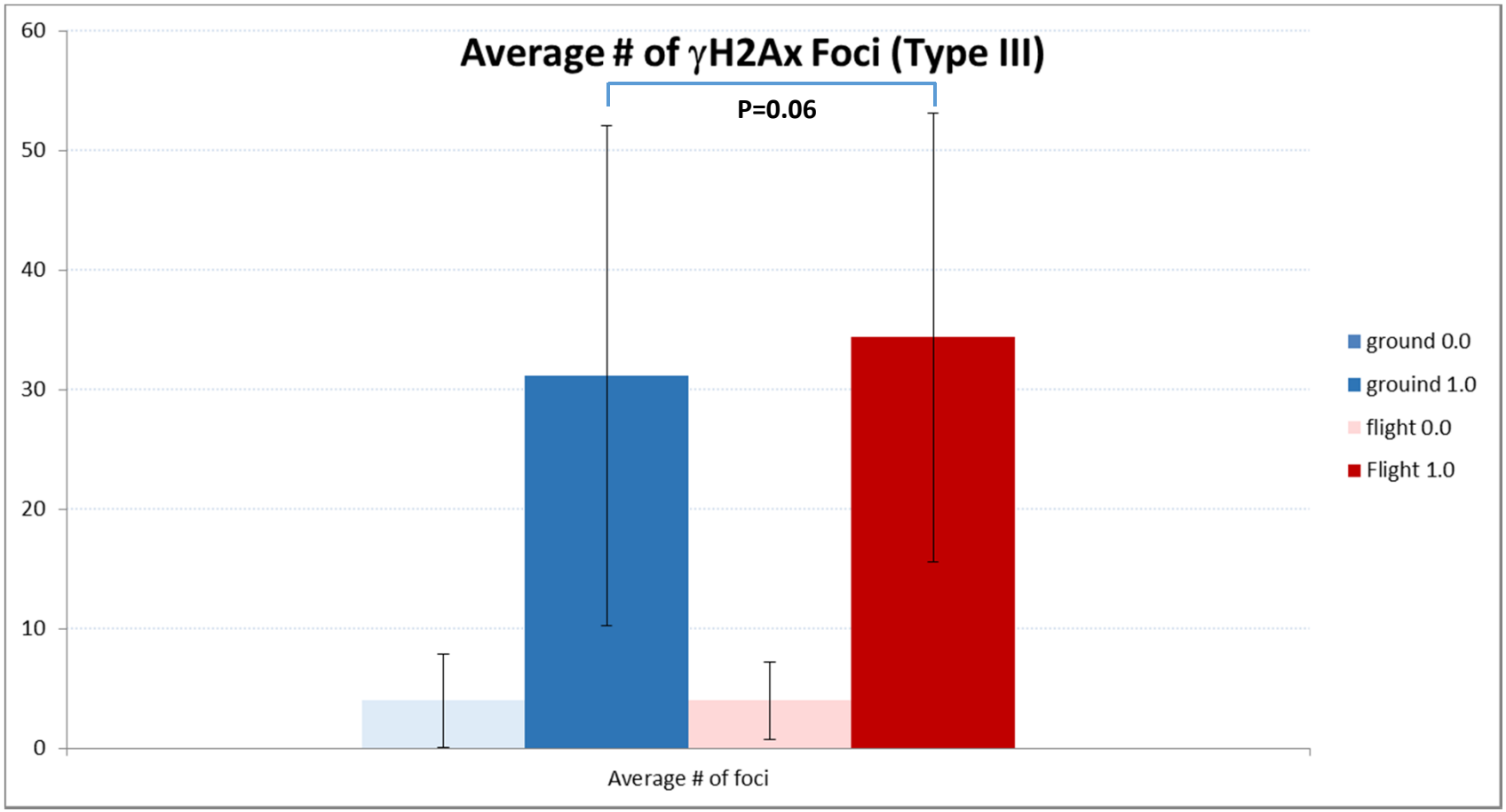


Damage in human fibroblasts will be measured by the phosphorylation of a histone protein H2AX after bleomycin treatment.

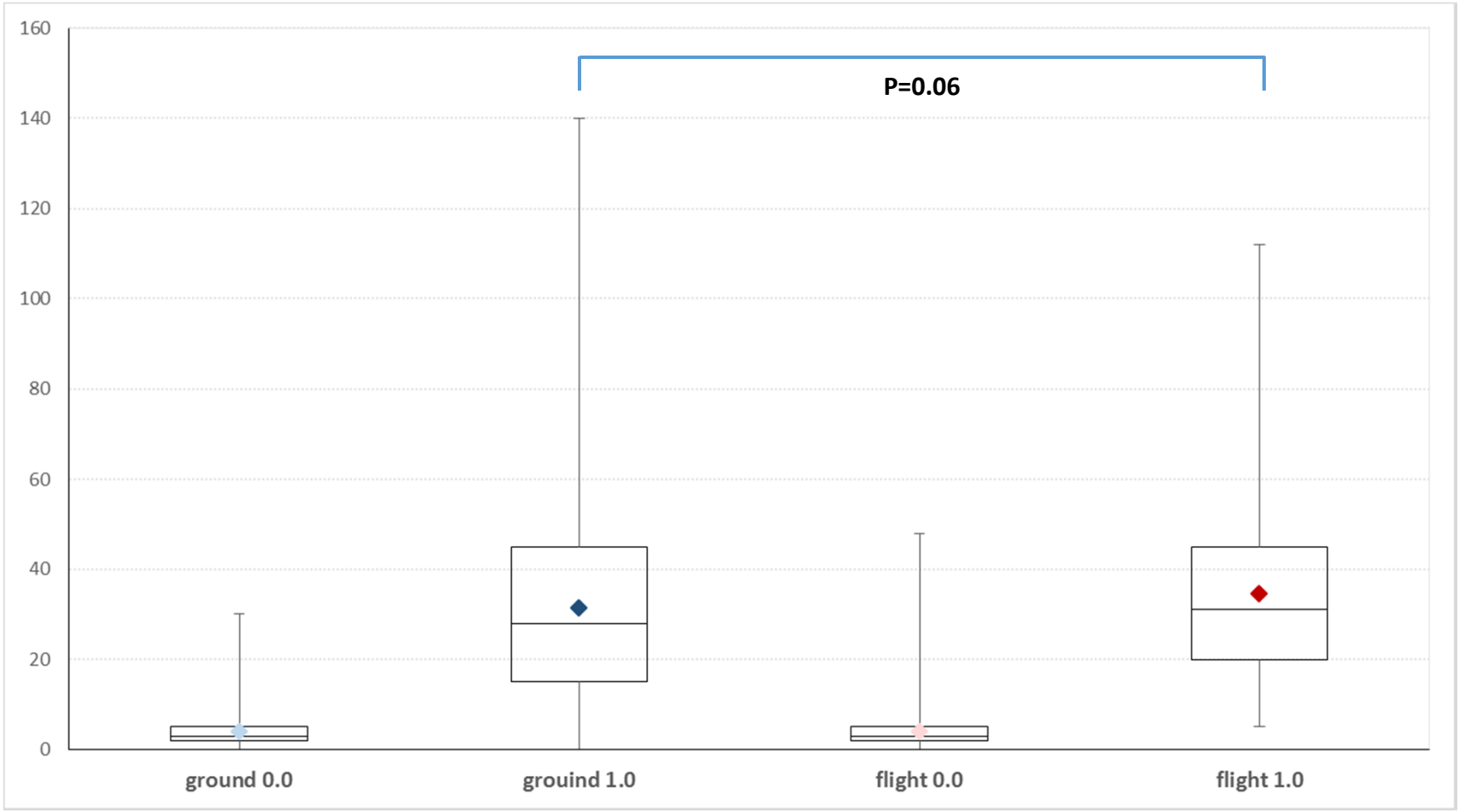

gH2Ax Staining pattern
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Type III
Type II
Type I
Placebo
Blemycin
Placebo
Bleomycin
Ground
Flight


γH2Ax Foci Count Histogram
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5
10
15
20
25
30
35
40
45
50
More
Ground 0.0
Ground 1.0
Flight 0.0
Flight 1.0

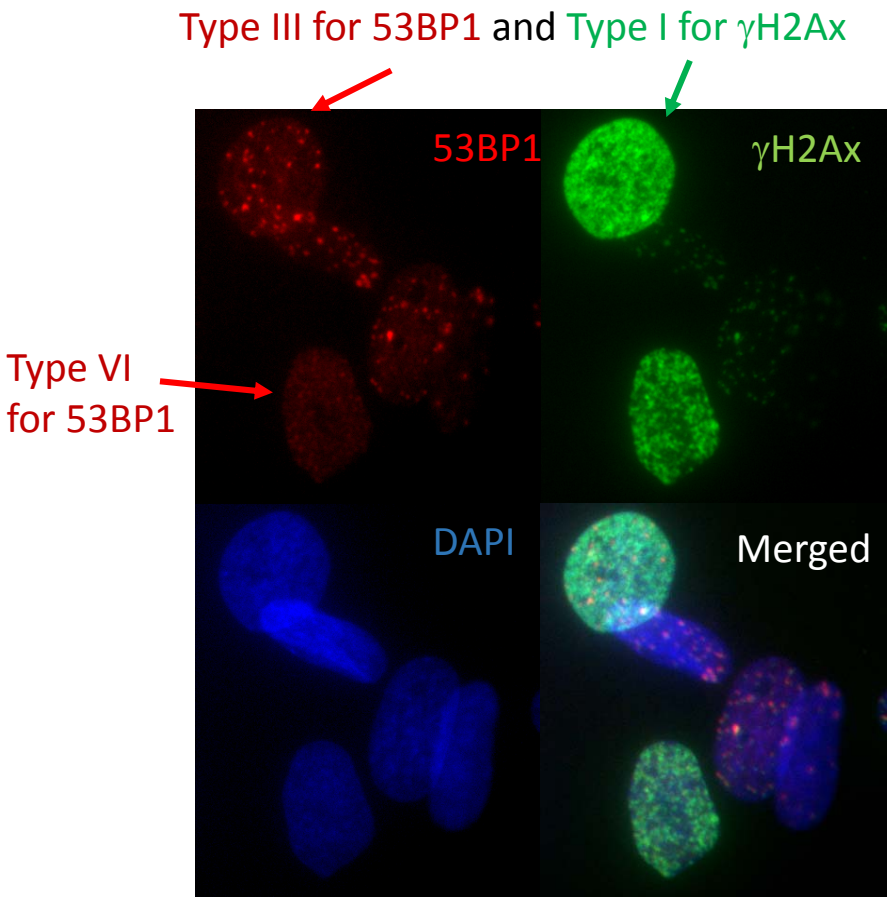

53BP1 Foci Count Histogram
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5
10
15
20
25
30
35
40
45
50
More
Ground 0.0
Ground 1.0
Flight 0.0
Flight 1.0

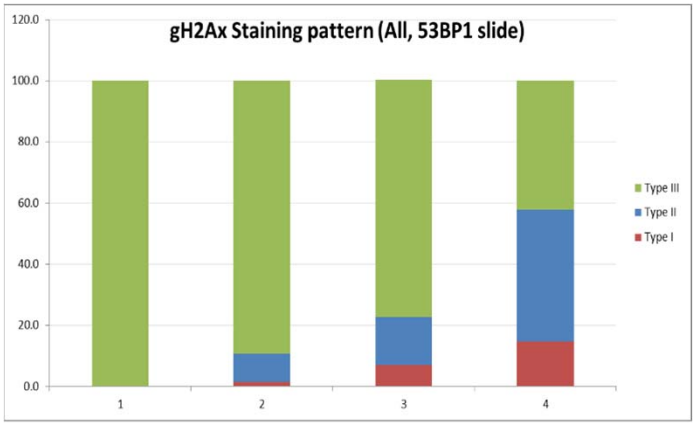

Average # of γH2Ax Foci (Type III)
P=0.06
60
50
40
30
20
10
0
Average # of foci
ground 0.0
grouind 1.0
flight 0.0
Flight 1.0

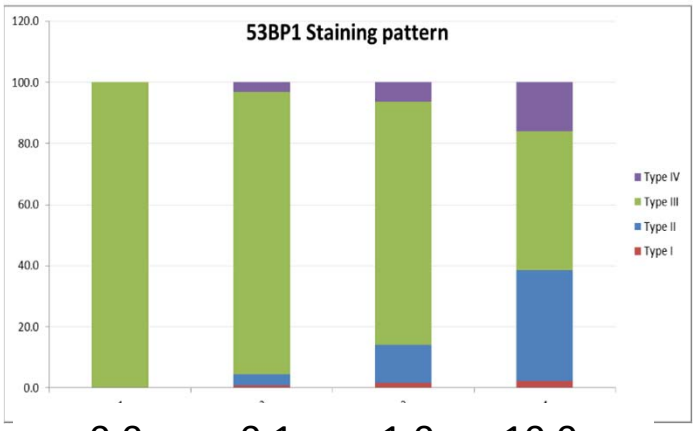
Average # of gH2Ax Foci (Type III)



Quantification of DNA Damages with 53BP1 Immunofluorescence Staining Patterns and Foci Counts

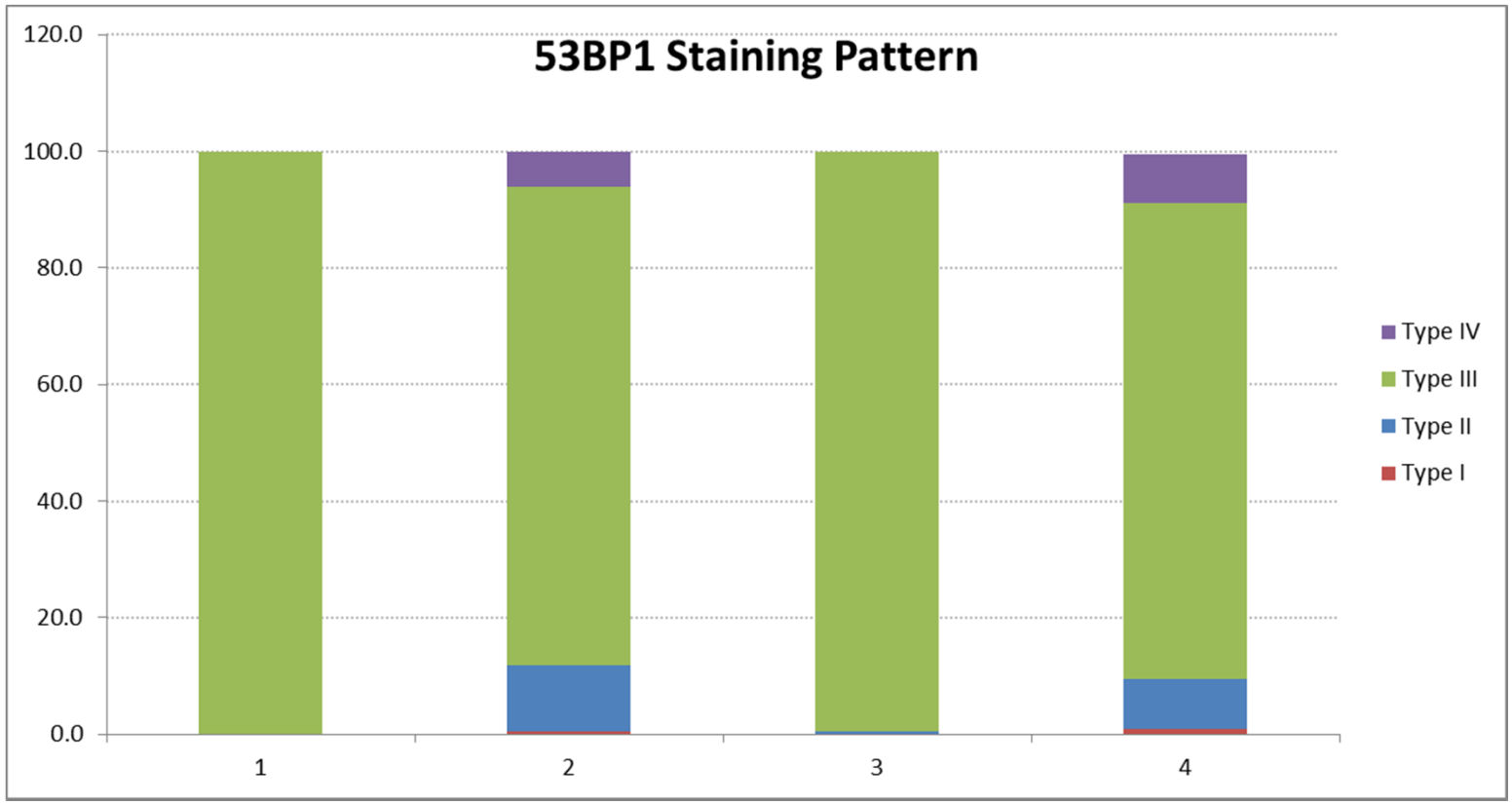


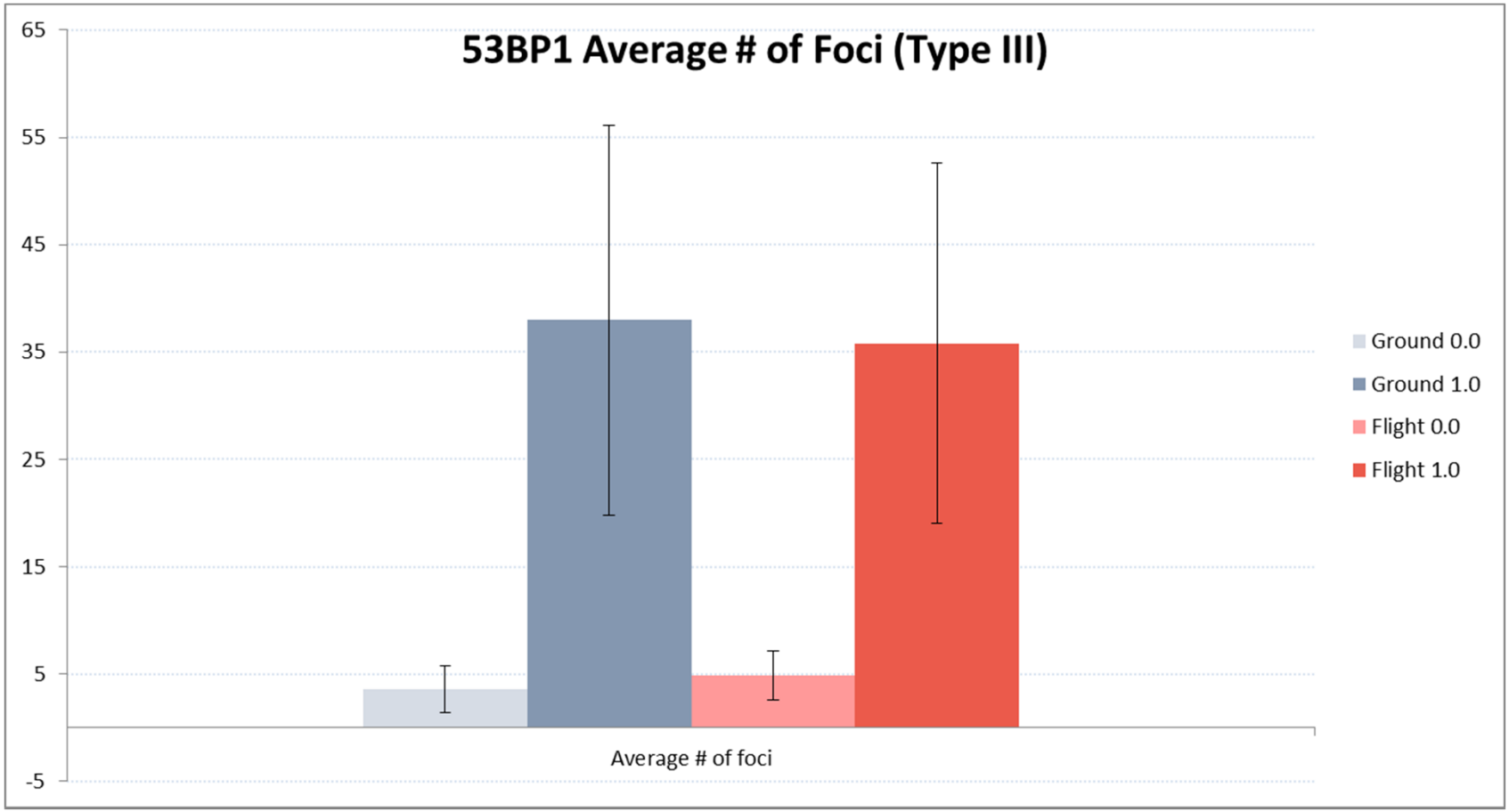


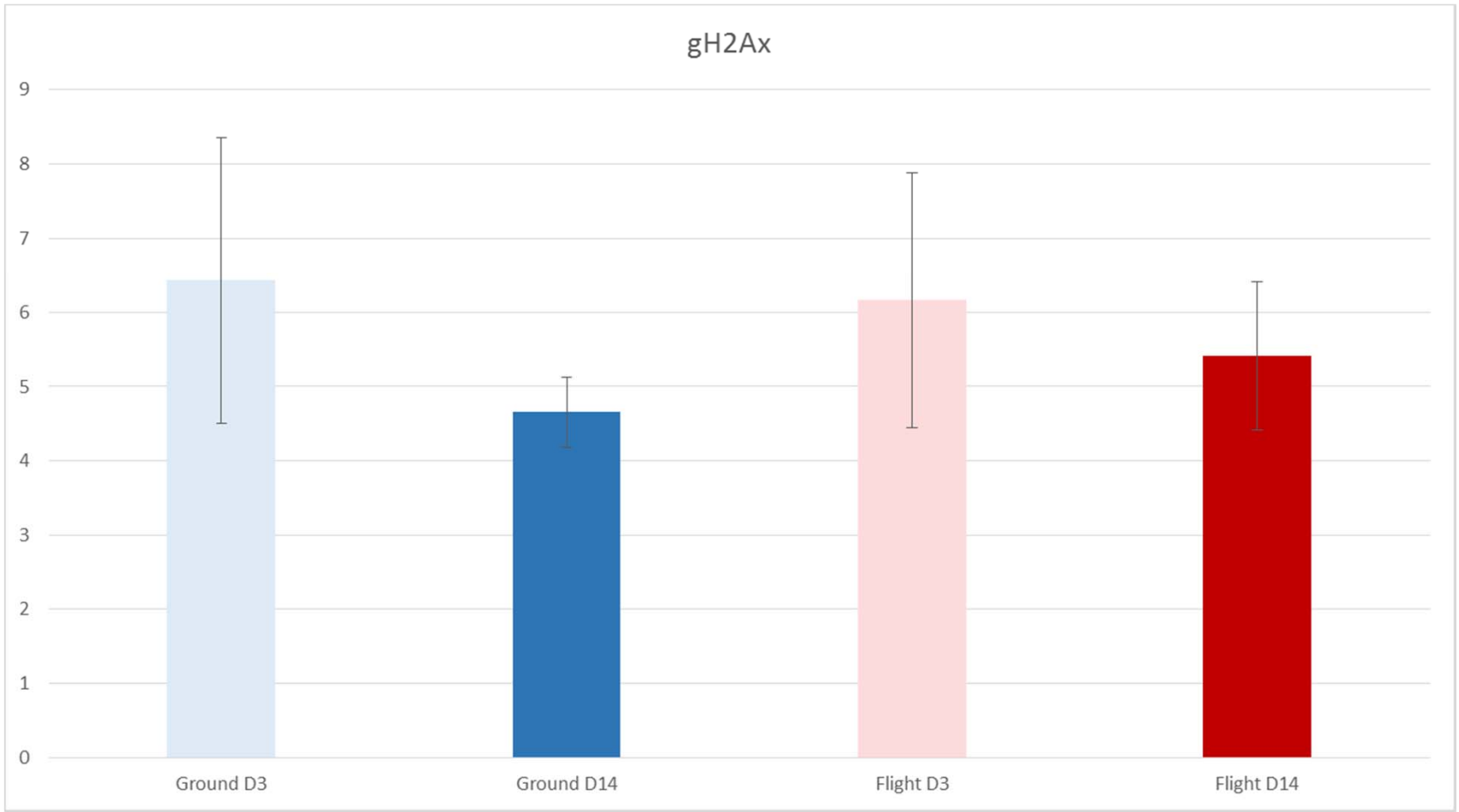


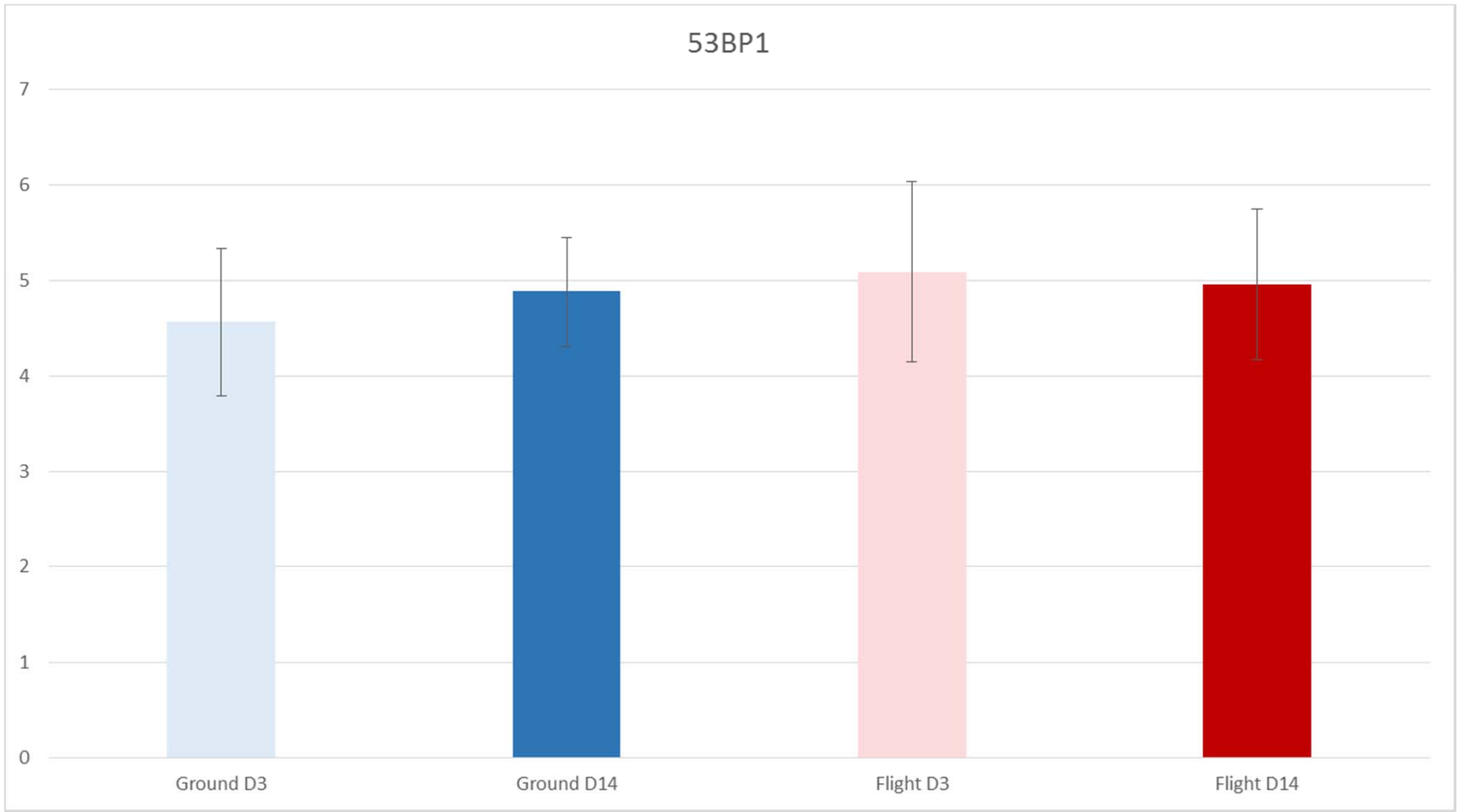
0.0 0.1 1.0 10.0

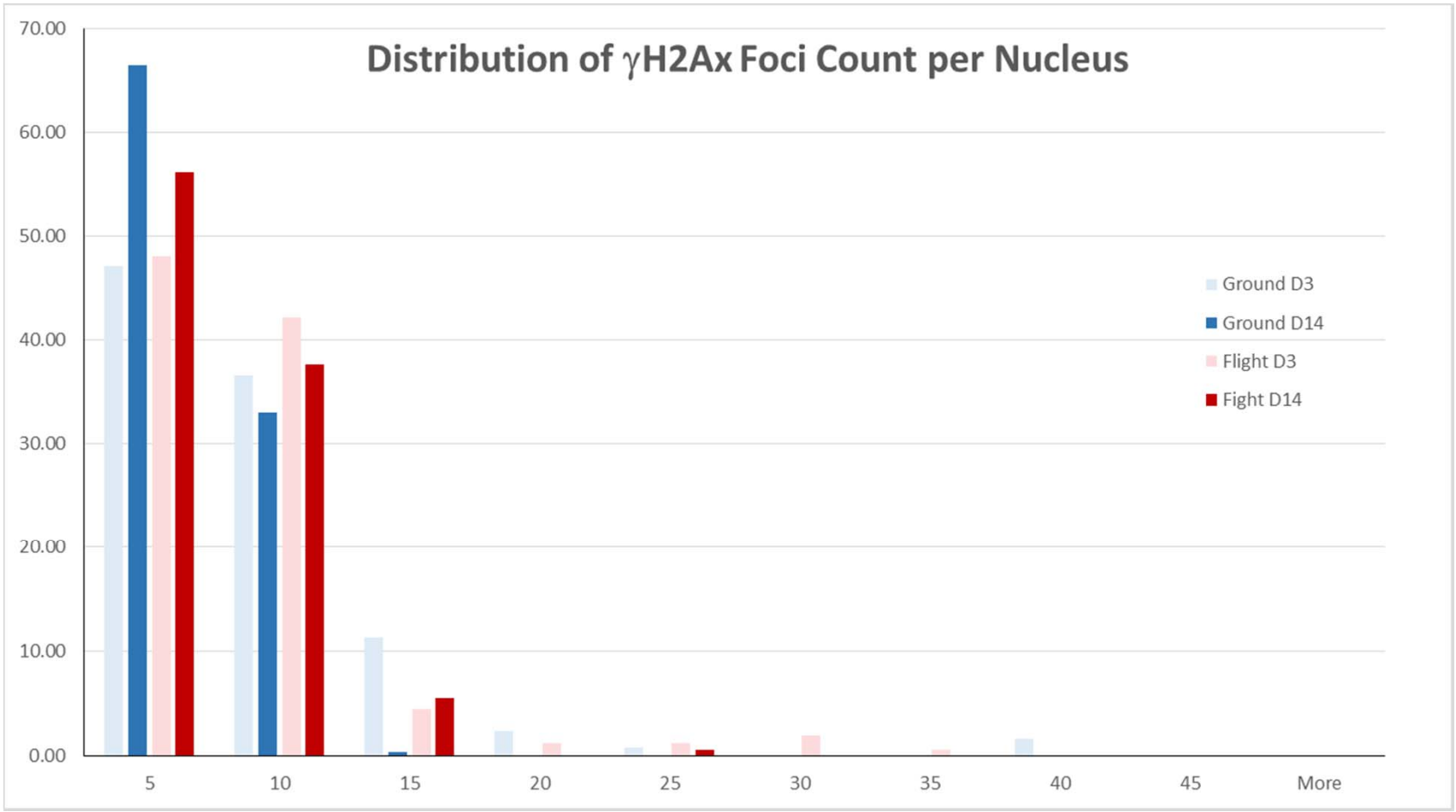
[Bleomycin], μg/ml

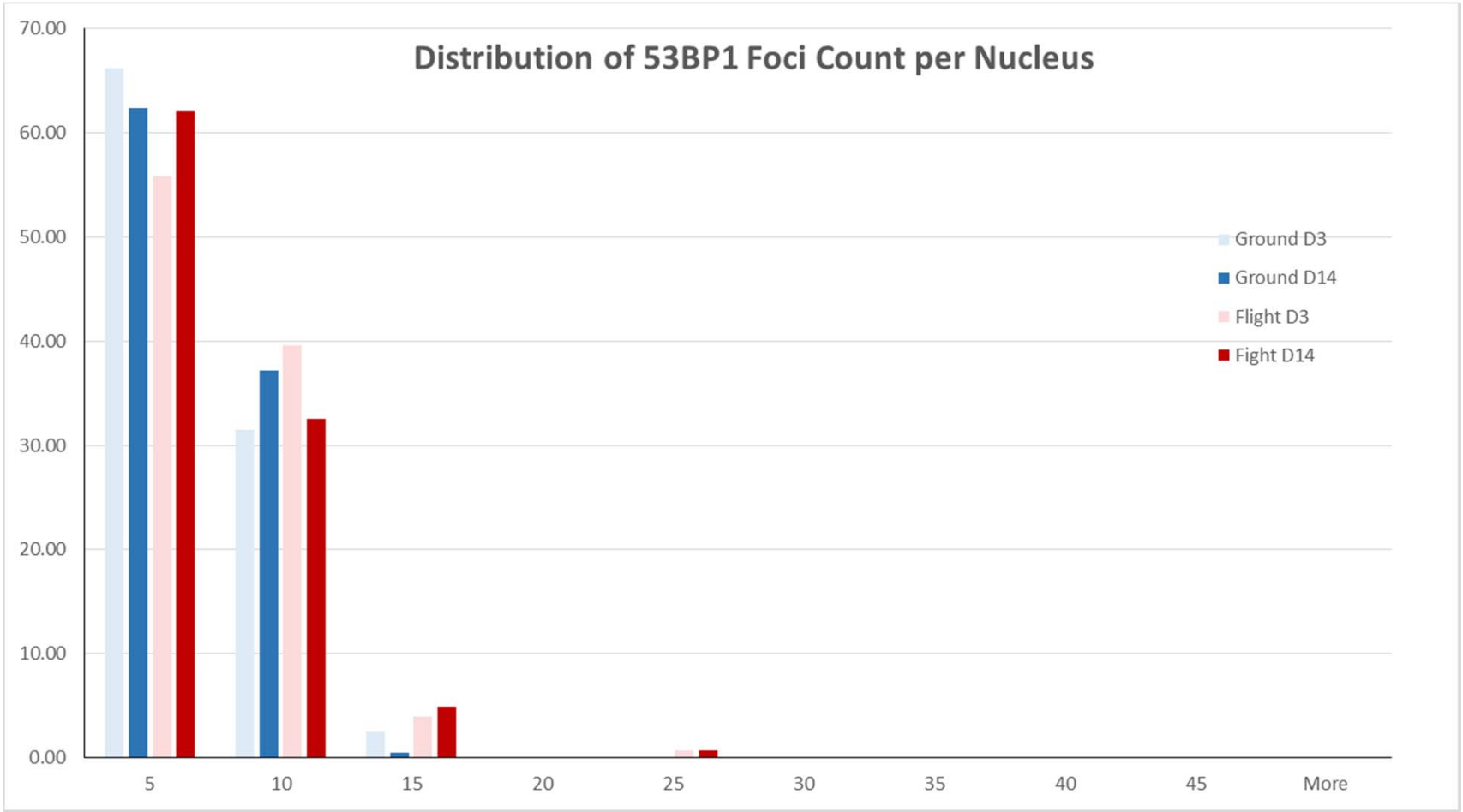

53BP1 Staining Pattern
120.0
100.0
80.0
60.0
40.0
20.0
0.0
1
2
3
4
Type IV
Type III
Type II
Type I


53BP1 Average # of Foci (Type III)
65
55
45
35
25
15
5
-5
Average # of foci
Ground 0.0
Ground 1.0
Flight 0.0
Flight 1.0

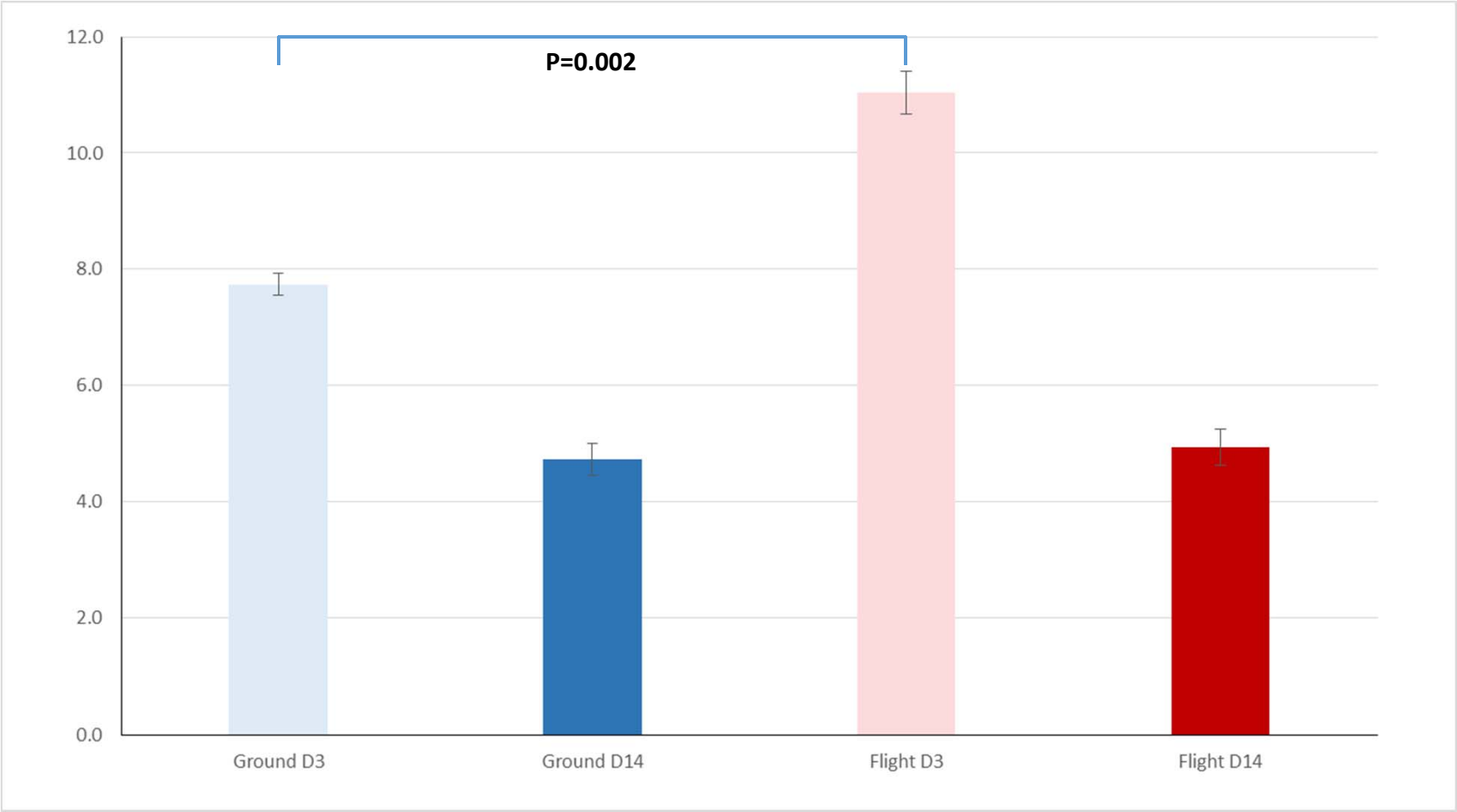

gH2Ax
9
8
7
6
5
4
3
2
1
0
Ground D3
Ground D14
Flight D3
Flight D14


53BP1
7
6
5
4
3
2
1
0
Ground D3
Ground D14
Flight D3
Flight D14

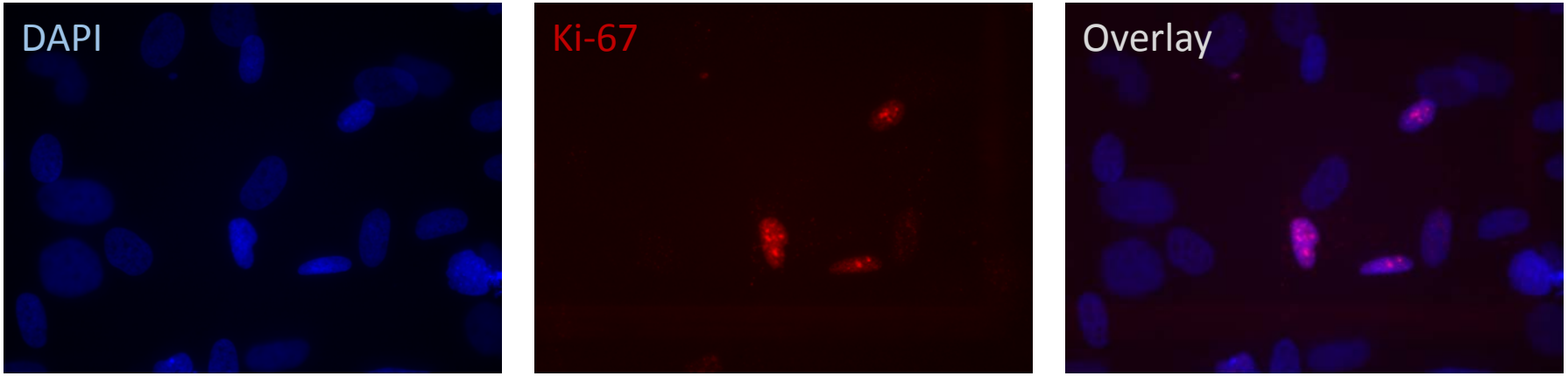

Distribution of γH2Ax Foci Count per Nucleus
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5
10
15
20
25
30
35
40
45
More
Ground D3
Ground D14
Flight D3
Fight D14


Distribution of 53BP1 Foci Count per Nucleus
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
5
10
15
20
25
30
35
40
45
More
Ground D3
Ground D14
Flight D3
Fight D14

Percent of Ki-67 Positive staining cells.



Cell proliferation marker – Ki67



CONCLUSIONS

- **Human fibroblast cells in the G1 phase of the cell cycle were flown on ISS for 3 and 14 days. Microarray analysis of gene expressions did not show a significant difference between the flight and the ground samples for either of the days.**

- **The gene expression patterns were significantly different between the 3 and 14 day samples, due potentially to the slow growth of the cells.**

- **On Day 3 after reaching the orbit, the cells were exposed to bleomycin to induce DNA damages. The degree of damages, as measured with immunohistochemistry staining for the induction of gamma-H2AX and 53BP1, did not show a significant difference in the response between the flight and ground samples.**

Acknowledgement

NASA Johnson Space Center
Ye Zhang
Tao Lu

NASA Ames Research Center
Fathi Karouia
Kevin Sato

BioServe Space Technologies
Louis Stodieck
Stefanie Countryman
Jonathan Beno

UT Southwestern Medical Center
Michael Story